METHOD OF CULTURING EUKARYOTIC CELLS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a continuation of U.S. patent application Ser. No. 13/383,829, filed on Sep. 10, 2012, which is a 371 of International Application No. PCT/US2010/041082, filed on Jul. 6, 2010, which claims benefit of U.S. Provisional Application No. 61/223,313, filed on Jul. 6, 2009, the entire contents of which are incorporated by reference herein in their entireties.

FIELD OF THE INVENTION

[0002] The invention relates to an apparatus and method for culturing eukaryotic cells in a bicarbonate-containing medium that allows maintenance of pH of the cell culture without the addition of bases directly to the culture medium.

BACKGROUND OF THE INVENTION

[0003] The culturing of cells for cell banking, for production of cell products, such as recombinant protein production is hampered by changing conditions as cells grow. While stainless steel bioreactors are often used for cell production, disposables are increasingly used at all stages in biologics manufacturing (Rao et al., 2009). In upstream processing, disposable bioreactors offer many advantages over their stainless steel counterparts (ranging from reducing cross-contamination risks to cost and time savings). The WAVE BIOREACTOR™ is a well-documented example of disposable upstream technology used for recombinant protein production in the biopharmaceutical industry (Cronin et al., 2007; Haldankar et al., 2006; Ling et al., 2003; Ye et al., 2009).

[0004] The WAVE BIOREACTOR™ system, as developed by Singh (Singh, 1999), comprises a pre-sterilized, flexible and disposable culture chamber (CELLBAG™), CO_2- and/or O_2-air mix controllers, and a pneumatically-controlled platform for rocking and heating the CELLBAG™. The rocking motion generated by this platform provides mixing and gas transfer in the CELLBAG™.

[0005] The WAVE BIOREACTOR™ system can be further equipped to provide online pH and dissolved oxygen (DO) monitoring and real-time feedback control (Mikola et al., 2007; Tang et al., 2007). However, the additional devices required, as well as the need for specially-designed bags to accommodate the pH and DO probes, increase the operational cost and complexity of this system. In addition, the base addition required to raise culture pH to the defined setpoint in pH-controlled bioreactors increases the culture osmolality. Depending on the extent of the osmolality increase in the bioreactor, the associated decrease in cell growth and viability (deZengotita et al., 2002; Zhu et al., 2005) may offset the benefits of pH control. In addition, if the pH probe malfunctions, the resulting pH perturbations may alter cell metabolism and promote cell death (Miller et al., 1988; Osman et al,, 2002).

[0006] Tight pH and DO controls may not be necessary for certain cell culture applications, such as, for example, the routine passage of cells in small-scale culture systems, such as shake flasks and spinners, for cell maintenance and expansion. However, pH and DO extremes are detrimental to cell growth and viability (Lin et al,, 1993; Link et al., 2004; Miller et al., 1988; Osman et al., 2001), and may affect product quality (Restelli et al., 2006; Yoon et al., 2005). Therefore, it is critically important to maintain some control over such growth conditions of cells for all stages of biologics manufacturing. Researchers previously demonstrated success in culturing CHO cells in a pH range of 6.8-7.3 and in the DO range of 10-100% of air saturation (Link et al. 2004; Restelli et al. 2006; Trummer et al. 2006; Yoori et al. 2005).

[0007] The added features of conventional bioreactors such as real-time pH monitors and DO monitoring control add significantly to the cost and labor-intensity of cell culture in biological manufacturing. Further, the failure or malfunction of these features can cause unacceptable variations and potential loss of the cell culture which is very costly in time and resources.

[0008] Thus, there is a need for improved methods for culturing eukaryotic cells without the need for introduction of strong bases, and without additional monitoring and real-time control of pH and DO.

SUMMARY OF THE INVENTION

[0009] The invention provides an apparatus and method to maintain pH in a cell culture system without the addition of base. In a bicarbonate-containing cell culture medium, the amount of CO_2 in the medium affects the pH of the medium, based on the carbonic acid-bicarbonate buffer equilibrium (Equation 1):

$$CO_2+HOH\text{<===>}H_2CO_3\text{<===>}H^++HCO_3^{31}$$

$$pH=pK-\log([CO_2]/[HCO_3^-])$$

Thus, the invention exploits this relationship to adjust the pH of cell culture medium without the need for addition of strong acids or bases by increasing or decreasing the dissolved CO_2 concentration using the dynamic interface of a liquid phase and gas phase of a cell culture system. The invention provides a method for achieving this modulation and an apparatus for practicing the method.

[0010] In general, the apparatus of the invention is supplied with air, oxygen or a combination of these gases to maintain the dissolved oxygen of the cell culture. By providing a gas mixture (which may be manipulated in terms of its composition and the rate of introduction) to the head space of the apparatus, CO_2 can either be added to or removed from the cell culture medium depending on the differential concentration of CO_2 between the liquid and gas phase. The removal of CO_2 from the head space will increase the culture pH as dissolved CO2 in the medium will diffuse out into the head space. Conversely, when CO_2 is added to the apparatus at a concentration that is higher than that of the medium, the CO_2 will dissolve into the medium and the culture pH will decrease. This invention provides a method that allows CO_2 transfer into and out of the cell culture to maintain culture pH without addition of base.

[0011] Thus, the invention provides a method for culturing eukaryotic cells comprising eukaryotic cells in a bicarbonate-containing culture liquid in a vessel, wherein the vessel has walls that encapsulate the cell culture and a gas phase head space above said cell culture. The vessel also comprises at least one port that provides an entrance and an egress of gas from said head space. The vessel is agitated to provide a dynamic interface between the liquid phase and the gas phase. The pH of the culture may be monitored and